Please add the following new claims:

--35. The method of claim 33 wherein said microspheres contain a gas including perfluoropropane.--

REMARKS

Claims 15-34 are pending; of these, claims 15-29 have been withdrawn from consideration, claims 30-34 stand rejected, and new claim 35 has been added. Applicant requests reconsideration and re-examination on the basis of the above amendments to the claims, the comments herein, and the accompanying declarations.

Applicant affirms the election of the gas species perfluoropropane and the material species human protein. Claims 30-34 and new claim 35 are readable on the gas species perfluoropropane and claim 31 is further readable on the material species human protein.

The above election has been made solely for purposes of convenience in commencing examination of the application. Should the Examiner find the elected claims allowable, he is respectfully requested to commence examination of the non-elected claim in the application.

Applicant is also submitting a supplemental Information Disclosure Statement with respect to references cited in corresponding cases which are not yet of record herein.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 30-34 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter not adequately described in the specification.

At the outset, Applicant notes that claims 32-34 have been amended. The amendments to claims 32 and 33 are for purposes of clarification, the term "suspensions" more accurately describing enhancing agents comprising liposomes or microspheres than the term "solutions." Amended claim 34 is now particularly directed to an enhancing agent that further comprises "a suspension of crystals in a saccharide diluent." Support for the claim is found at page 15, lines 1-12 of the specification.

Applicant further notes that each of dependent claims 31-34 depends from independent claim 30, which is directed to an improved ultrasound imaging method comprising "enhancing the contrast of an ultrasound image by selecting for use as an enhancing agent microbubbles of a gas including perfluoropropane." (See Response filed 7/9/98). In comments regarding claims 32-34, the Examiner appears to have considered each dependent claim as depending from the immediately preceding claim. For example, the Examiner characterizes claim 31 as being "drawn to a composition comprising human protein and perfluoropropane" and then further states that Claim 32 "adds the additional ingredient liposomes." Claim 32 however depends from claim 30 and does not require the combination of human protein and liposomes. Likewise, in referring to claim 33, the Examiner uses the language "the further addition of

microspheres." Claim 33 however also depends from claim 30 and does not require the combination of human protein and liposomes and microspheres.

Applicant further notes that in rejecting claim 30 under 35 USC §112, first paragraph, the Examiner has provided no reason for the rejection of claim 30. Rather the Examiner acknowledges that the Applicant's disclosure teaches that perfluoropropane may be used as gas. Applicant respectfully submits that the rejection as to this claim should be withdrawn.

The Examiner has set forth reasons for rejecting claims 31-34, under 35 USC §112, first paragraph, and each claim will be addressed in turn. In addressing whether the written description requirement of 35 U.S.C. §112, first paragraph, is met, the Examiner is reminded that an explicit disclosure of claim language has never been required. In re Wertheim established that lack of literal support is not enough to warrant a rejection under § 112. 541 F.2d 257, 265 (CCPA 1976). Further, an applicant need not describe the claimed invention in the same, exact words in order to meet the written description requirement. Eiselstein v. Frank, 52 F.3d 1035 (Fed. Cir. 1995). Rather, the disclosure need only reasonably convey to persons skilled in the art that the inventor had possession of the claimed subject matter as of the filing date sought, "even if every nuance of the claims is not explicitly described in the specification." In re Alton, 76 F.3d 1168, 1175 (Fed. Cir. 1996). The disclosure here reasonably conveys that the inventor had possession of the claimed subject matter of claims 31-34.

In the present case, applicant's disclosure teaches a method whereby one skilled in the art can "select particular gases based on their physical and chemical properties for use in ultrasound imaging." (see, e.g., page 20, lines 25-28). As the Examiner has acknowledged, one such gas is perfluoropropane. Once the gases are selected, applicant's disclosure further specifically teaches that by "[u]sing existing techniques, substantially improved contrast-enhancing media may then be produced and used to improve the quality and usefulness of ultrasound imaging" (page 21, lines 15-18). These teachings at least reasonably convey to one skilled in the art that applicant's invention includes the selection of specific gases, e.g., perfluoropropane, and the use of the selected gases to prepare contrast media using existing techniques.

While a person skilled in the art could take this teaching of "existing techniques" by itself to understand that the claimed subject matter was in the possession of the inventor, the specification provides with further particularity the meaning of "existing techniques." In this regard, the Examiner is referred to the text at pages 6 through 20 of the specification. These sections, entitled "Techniques for Measuring Ultrasound Contrast-Enhancement Phenomena" and "The Materials Presently Used as Contrast Enhancing Agents" survey types of ultrasound contrast media which were under study at the time this application was filed. Based on this disclosure, the application at the least reasonably conveys to one skilled in the art that the Applicant was in possession of the claimed gas perfluoropropane

in combination with any of the described ultrasound contrast media under study at the time of filing.

Turning to claim 31 in particular, the Examiner acknowledges that the disclosure teaches the use of human protein and perfluoropropane but nevertheless indicates that the disclosure "fails to teach that the two components would be used in combination with one another." The Examiner further takes the position that the combination "amounts to a sub-genus which was not present in the original specification." However, this is not the case, nor is it the proper standard for determining whether one skilled in the art was in possession of the claimed invention. Again, that standard only requires that the specification reasonably convey this to those skilled in the art. As discussed above, perfluoropropane would be regarded as just one of the gases to which the invention pertains, and the particular claimed formulation would be regarded as among those contemplated for perfluoropropane, among the other inventive gases. Examples of existing techniques for the formulation of ultrasound imaging media that specifically includes the use of human protein is particularly described at page 17. Based on this disclosure, it is clear that applicant had possession of the presently claimed invention of claim 31 at the time of filing.

Similarly, it is equally clear that the applicant had possession of the claimed subject matter of claims 32-33 and new claim 35. The existing technique of using liposomes in contrast media is particularly described at pages 15-16. The

existing technique of using microspheres in contrast media is particularly described at page 13.

With respect to claim 34, the Examiner states that the disclosure "fails to teach that a powder would be present in the final composition." Claim 34 as amended no longer requires a "powder" element, and is now directed to an enhancing agent further comprising "a suspension of crystals in a saccharide diluent." As with the above claims, it is also equally clear that Applicant had possession of the claimed subject matter of this claim, as now amended. In particular, the existing technique of using a suspension of crystals in a saccharide diluent in contrast media is particularly described at page 15.

In light of the above, it is respectfully submitted that the rejection under 35 U.S.C. §112, first paragraph, should be withdrawn.

Rejection under 35 USC §112, second paragraph

Claim 33 is rejected under 35 U.S.C. §112, second paragraph, as being indefinite. In particular, the Examiner states that the claim is confusing because it is unclear what is meant by the term "microspheres." "Microspheres" are discussed at page 12 of the specification in the context of existing techniques of forming contrast media that use encapsulated air-filled microspheres. Applicants submit that one skilled in the art would readily understand the term, and respectfully request withdrawal of the rejection.

New claim 35 depends from claim 33 and further defines a composition in which the microspheres contain a gas including perfluoropropane. Applicant submits that this claim too meets the requirements of 35 U.S.C. §112, second paragraph.

Claim 34 is rejected under 35 U.S.C. §112, second paragraph, as indefinite. In particular, the Examiner has objected to the term "powder" as being indefinite, and to the combination of a powder with a diluent as being confusing. Claim 34 has been amended to overcome the rejection. Amended claim 34 now recites an enhancing agent further comprising "a suspension of crystals in a saccharide diluent." Support for the claim can be found at page 15 of the application. Applicant respectfully submits that amended claim 34 satisfies the requirement of 35 U.S.C. §112, second paragraph.

Rejection under 35 USC §103

Claims 30-34 stand rejected under 35 USC §103 as unpatentable over Rössling et al., Tickner, Schneider et al., Tickner et al., Glajch et al., Albayrak et al., and Swanson in view of Vygantas et al, Jacobs, the DuPont Technical Bulletin and references of Lincoff et al.

Applicant submits herewith additional declarations filed in reexamination proceeding No. 90/004,656, of U.S. Patent 5,558,094, which patent is related to the present application and in which some similar issues have arisen.

These declarations are:

Declaration of Steven C. Quay.
Declaration of Richard Stuart Meltzer.
Declaration of Donald J. Burton.
Declaration of Pamela Hilpert.
Declaration of Johnny Lai.
Declaration of Dean Kessler.
Second Declaration of Steven C. Quay.
Second Declaration of Donald J. Burton.
Second Declaration of Johnny Lai
Second Declaration of Dean Kessler.
Declaration of Sonia Maria Souza.
Declaration of Martin Lee.
Second Declaration of Pamela Hilpert.

In response to the Office Action: first, Applicant submits that the primary and secondary references are not combinable because the secondary references constitute non-analogous art, and that there is thus no prima facie case of obviousness. That these references are in fact non-analogous art is amply set forth in several declarations, mainly those of Hilpert and Burton, as discussed below.

Rössling et al. and its disclosure, or lack of the same, is discussed in the two Burton declarations. As recognized by the Examiner, Rössling et al. do not specifically name perfluoropropane (or the other particular gases of the current claims, perfluorobutane and perfluoropentane). Rössling et al. do disclose the use of "halogenated hydrocarbons or mixtures" as ultrasound contrast media and list five examples,

only one of which, dibromodifluoromethane, contains any fluorine atoms at all.

Professor Burton, in his first declaration, reviews the disclosure of Rössling et al. as a whole and states (paragraphs 13-16):

> "Rössling includes a long list of chemicals for use in an ultrasound contrast agent. One of the classes of chemicals mentioned by Rössling is 'halogenated hydrocarbons,' a very large class of chemicals (at least thousands) since it is unlimited as to the number of carbon atoms which might be included if it is considered alone.
>
> Rössling does list five halogenated hydrocarbons: methylene chloride; 1,1-dichloroethylene; isopropyl chloride; dibromodifluoromethane and bromomethane. Of these five compounds, only one includes any fluorine at all - dibromodifluoromethane (CF_2Br_2). None of the five halogenated hydrocarbons disclosed in Rössling are perfluorocarbons. I believe that one skilled in the art of chemistry in 1991 or 1992 would take the examples of halogenated hydrocarbons provided by Rössling to indicate a preference for low molecular weight (specifically one to three carbon) chlorine containing molecules, since most of the compounds listed include chlorine. In my experience the compounds listed were probably those identified by the patentee as commercially available at the time. Bromine and fluorine are also included, so it is possible that one skilled in the art might consider small (i.e. one to three carbon) molecules containing such atoms as well.

> Without knowing what is described in the '094 patent[1], I believe that one skilled in the art of chemistry in 1991 or 1992 would find no guidance at all in the chemicals listed by Rössling to even try a perfluorocarbon chemical as a component of an ultrasound contrast agent. At best, if for some reason the skilled chemist thought that fluorine containing chemicals might be useful, that person would have looked to the one fluorine containing halogenated hydrocarbon listed by Rössling -- dibromodifluoromethane -- for guidance. If so, the skilled chemist could possibly have thought that perfluoromethane might have value. However, even the possible selection of this compound is unlikely if the further discussion of preferred gases by Rössling is considered. That is, since Rössling states that the compounds preferred for use in his contrast agents are air, argon, xenon, sulfur hexafluoride, propane, butane and furan, and since none of these is a halogenated hydrocarbon (much less a perfluorocarbon) one skilled in the art is effectively directed away from choosing any halogenated hydrocarbon or perfluorocarbon compound."

Applicant submits that the first declaration of Professor Burton more than adequately treats the issues of the overall disclosure of Rössling et al.. However, Prof. Burton provided a second declaration for additional clarification.

In the second declaration Professor Burton further analyzes the Rössling disclosure, and states that the same conclusion is reached whether the analysis focuses on the individual chemicals listed as examples of "halogenated hydrocarbons" or whether the phrase "halogenated hydrocarbons" should be taken

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[1]The present application is a continuation of a division of said patent

in light of the listing of individual hydrocarbons elsewhere in the reference. He points out that at the time in question those skilled in the art were aware that the chemistry of perfluorocarbons was well known to be quite different than other (non-perfluorinated) halogen containing chemicals, and concludes that those skilled in the art would not have been directed in 1991 towards selecting the claimed compounds by referring to Rossling's list of hydrocarbons together with the use of the term "halogenated hydrocarbons".

Thus, Rössling et al. do not disclose or suggest to those skilled in the art to use perfluorocarbons and specifically perfluoropropane (and the other claimed perfluorocarbons), as ultrasound contrast agents.

Tickner '251 discloses the use of gas bubbles to measure pressure within a liquid containing vessel, particularly within a cardiovascular system. A number of gases are mentioned, including freons. While the class of freon gases does include two of the three claim-designated species, Dr. Burton's first declaration (paragraphs 7-11) points out that there are, at least, hundreds of such compounds and that the disclosure of Tickner includes a number of other non-halogenated gases, particularly nitrogen, oxygen, argon, xenon, air, methane, carbon monoxide and carbon dioxide, and teaches a preference for carbon dioxide. Tickner provides no reason to choose any one of the listed chemicals over another, provides no reason to choose freons over the other

types of gases mentioned, and provides absolutely no reason to choose particular freons for his purposes.

In contrast, Tickner et al. '885, which is copending with the '251 reference, contains no mention whatever of any halogenated gas, least of all a perfluorocarbon, for such purpose. Tickner et al., column 4, lines 16-29, speak about the gases in general, advising that they should be chemically inert and somewhat slowly dissolving, and mentions nitrogen and other noble gases and carbon dioxide.

Neither Tickner nor Tickner et al. therefore contains disclosure relevant to the current claims. Furthermore, the statements in the Burton declarations regarding what would be taught by use of the term "freons" are applicable to this reference as well as to Rössling.

Schneider et al. disclose gaseous microbubbles stabilized by converting surfactants used into lamellar or laminar form. Among the gases mentioned for use in the reference compositions are "freon and mixtures thereof".

As discussed more fully below, Applicant's claimed compound perfluoropropane (as well as claimed compound perfluorobutane and perfluoropentane) show surprising persistence in the body, and that property would not be obvious from the miniscule disclosure of "freon" in Schneider et al. Schneider et al. simply do not lead those skilled in the art to the use of the claimed compounds in ultrasound contrast agents,

particularly agents which can survive passage through the heart and lungs.

Bichon et al., U.S. Patent 5,711,933, newly submitted herewith, contains a disclosure of "freon" among a number of other ingredients, (col. 6), and the same comments apply to this reference as for Schneider et al.

While the Burton declarations do not specifically comment on the disclosure of Schneider et al. or Bichon et al., their comments on what would be disclosed by use of the term "freon" or "freons" is relevant to these references as well. (In addition the DuPont Technical Bulletin states that the term "freons" as used therein is limited to molecules having up to four carbon atoms, so does not encompass the claimed perfluoropentane).

Glajch et al. is cited as teaching microparticles containing a gas for ultrasonic imaging and specifically mentioning perfluoromethane and perfluoroethane (column 6, line 62). Glajch et al. thus do not disclose use for ultrasonic imaging of the perfluorocarbons presently claimed. The Glajch et al. disclosure also states that "other light gases" are expected to provide useful ultrasound contrast properties. However, the Burton declarations point out that this would simply teach those skilled in the art to use other low boiling point, high volatility gases as ultrasound contrast agents, but would not teach them to use the claimed perfluorocarbons for this purpose.

Albayrak et al. similarly contains no disclosure relevant to or leading to the concept of the use of perfluorocarbons in ultrasound imaging.

Swanson et al. cite a statement supposedly made in another reference, of Zisken et al., that materials which are liquid at room temperature and vaporize at body temperature (including ether and "perfluorocarbon") may represent effective intravascular ultrasonic enhancement agents providing they are non-toxic. However, the Zisken et al. reference relied on for this statement contains no mention whatever of perfluorocarbons. Swanson et al. thus is not an effective reference of this information and is not an effective reference against the claims. Otherwise, Swanson discloses only information relevant to perfluorodecalin, and which is not relevant to the claims in this Application.

The secondary references, as the examiner recognizes, do not relate to ultrasound imaging. The Examiner submits that they are relevant and would be considered by those skilled in the art of ultrasound imaging based on various teachings including the importance and usefulness of small perfluorocarbon gases in vivo and the long persistence of their effect due to their stability and insolubility. Applicant does not agree with this characterization.

This issue is addressed in the declarations of Dr. Hilpert. In those declarations Dr. Hilpert states that the problem to which the Lincoff and Vygantas references were addressed involves a need for improving therapeutic treatment for retinal

detachment by injection into the eye of the subject, of a single large bubble. The bubbles in the Lincoff references are from 0.4 to 0.4 cc; in Jacobs bubbles of 0.5 mL are indicated as being approximately 2000 microns (2 mm) in size. These are of orders of magnitude different from the claimed bubbles, which must be at most about 8-10 microns in order to be used for imaging in the cardiovascular system.

In her second declaration Dr. Hilpert points out that the problems facing those seeking to develop contrast agents in 1991 (the priority date of the present application) were completely different than those being addressed by the authors of the secondary references. On the basis of the information in her declarations she concludes that those skilled in the art of ultrasound contrast agents would not have consulted or considered these secondary references in seeking and arriving at solutions for the problems involving development of new ultrasound contrast agents.

Dr. Hilpert also points out (second declaration, paragraph 4);

> "In addition, the vitreous of the eye is a poor approximation of an animal's cardiovascular system and therefore the value of these teachings to one studying the problem of developing ultrasound contrast agents is accordingly diminished. For example, the viscosity of the vitreous is high compared to blood, which is likely to change the rate that gases diffuse. The pressure of the arterial beds is high and pulsatile, and different from the non-vascular vitreous compartment of the eye. The partial pressure of gases in the blood changes dramatically as the blood moves through the venous system, the lungs, the

> arterial system and the systemic capillaries, unlike in the non-vascular compartment of the eye. Finally, the solubility of a given gas is likely to be quite different in blood than in the eye and thus the predictive value of the teachings is further diminished."

In addition, this issue is also addressed in the Meltzer declaration. Dr. Meltzer describes approaches taken by the prior art and those skilled in the art in 1991 toward solving the problem of obtaining ultrasound contrast agents with good persistence and the ability to survive passage through the pulmonary bed. Dr. Meltzer points out (paragraph 13) that those skilled in the art were pursuing avenues of research other than determining the optimum gas to be used in these agents.

He goes on to discuss the secondary references cited herein and states that he "cannot fathom that a person of ordinary skill in the art in September 1991 time frame would have understood that their disclosures would have any application in the relevant field of endeavor, i.e., ultrasound contrast agents." He also points out that these references are not in any analogous art but in a different field, namely ocular treatment; that they disclose the use of relatively very large gas bubbles for retinal attachment, and address problems which have no relation to the problem of development of persistent ultrasound contrast agents which could survive passage through the pulmonary bed.

Applicant submits these declarations clearly indicate that those skilled in the art would not look

to these secondary references for information useful or relevant to producing improved ultrasound contrast agents; thus no prima facie case of obviousness is made. It simply would not have been obvious to those skilled in the art to combine the secondary references with the primary ones. As pointed out in the Hilpert declarations those seeking to develop improved ultrasound contrast agents were looking to identify better or different materials which could be used to encapsulate or carry the gas microbubbles as opposed to seeking different gases for use in such agents. The primary references cited by the Examiner support this conclusion in that little or no attention is given to fluorinated materials, and virtually none to any perfluorocarbons, for use in such agents. Those skilled in the art, therefore, not being particularly interested in perfluorocarbons for use as ultrasound imaging agents in the first place, would certainly not have looked to the non-analogous secondary references for more information about these materials.

Applicant therefore submits that the cited references, taken in the combinations as proposed, do not provide a prima facia case of obviousness.

Even if, for the sake of argument, it is assumed that a proper prima facie case of obviousness has been made out, Applicant submits that such case is fully rebutted by the declarations submitted herewith, which show unexpectedly superior performance in ultrasound imaging for the claimed compound perfluoropropane (as well as other compounds

of the claims herein, namely, perfluorobutane and perfluoropentane).

The evidence relied on in support of patentability, should that be necessary, consists of the two Quay declarations, the two Lai declarations, the two Kessler declarations, the Souza declaration (which contains statistical analysis of the results in the work by Kessler), the Lee declaration (reviewing the approach by Dr. Souza in analyzing the data) and portions of the second Hilpert declaration.

In addition the declarations of Meltzer and Hilpert (first declaration) are considered to establish the existence of a long felt but unsolved need in 1991 for an ultrasound contrast agent with sufficient persistence to allow effective imaging of myocardial perfusion, a marker of coronary heart disease.

The Quay declarations show measured persistence values for two of the claimed compound perfluoropropane (as well as the claimed compound perfluorobutane), in comparison with prior art gases air, perfluoromethane, perfluoroethane and difluorodibromomethane. The highest measured persistence value of a prior art gas was that for perfluoroethane, which was measured at 9.8. On the other hand the two above-mentioned claimed compounds have measured persistence values of 51.2 and 244, respectively. The lowest of these is more than five times that of the closest structure prior art compound. In addition, the determined persistence values correlate to the calculated Q values of these

materials contained in this application in that the compounds having the largest calculated Q values also have the largest experimental Q or persistence values. This would not have been expected from any information known about these compounds in the prior art and is thus strong evidence of non-obviousness of this invention.

The Lai declarations provide detailed laboratory data from in vitro studies in which actual persistence was measured, as well as (in the first declaration) the size of microbubbles formed. The Kessler declarations provide detailed laboratory data from in vivo studies of persistence. These declarations demonstrates that formulations according to the claimed invention yielded markedly superior persistence over prior art gases.

In a related proceeding, the Examiner expressed concerns with regard to the first declarations of Hilpert, Lai, and Kessler, and the two Quay declarations. The Examiner's specific concerns were:

1. The measurement of persistence was not done by optimal means.
2. Multiple samples of formulations were not tested.
3. Multiple measurements with each formulation and statistical analysis of the data were not performed.
4. The role of microbubble size was not satisfactorily taken into consideration.
5. Tests were conducted in vitro rather than in vivo.

6. The tests did not show unexpected results.

The second declarations of Hilpert, Lai and Kessler, and the declarations of Lee and Souza are believed to address the Examiner's concerns and sufficiently support unobviousness and patentability of the claims in this application. These declarations provide data which (1) provide in vivo data which additionally is consistent with the in vivo and in vitro data presented earlier; (2) include measurement of utility as contrast agents by an art-recognized method using indicator-dilution curve analysis of Area-Under-the-Curve data; (3) include measurements which are statistically reliable, and (4) unequivocally show superior and unexpected performance of the claimed compound perfluoropropane (as well as pefluorobutane (decafluorobutane) and dodecafluoropentane (perfluoropentane)) over the closest prior art materials.

The second Lai declaration describes preparation of some of the materials used in the second Kessler declaration. The second Kessler declaration (Kessler II) describes a number of in vivo tests of several different formulations of claimed and prior art compounds, with measurement of the area-under-the-curve (AUC) values, averaging replicates for each combination of gas and carrier and determining the standard error of the measurement (paragraph 7). Dr. Kessler also performed a similar analysis on the results of experiments described in his first declaration (see second Kessler declaration, Table 1).

Figures 1-4 of Kessler II show four exemplary AUC curves from which can be compared the plots for the raw data of some of the experiments, namely albumin stabilized microbubbles of perfluoroethane (Figure 1) and perfluoropropane (Figure 2) and surfactant stabilized microbubbles of the same compounds (Figures 3 and 4). The comparisons are done in a manner in which all other potential variables remain constant and show the AUC values, i.e., persistence, between the prior art compound perfluoroethane and the claimed compound perfluoropropane.

Additional data including comparisons to the other claimed compounds - perfluorobutane and perfluoropentane - is found in Table 2 of Kessler II, which is reproduced below for convenience.

Table 2

Summary of Area-Under-Time-Intensity Curve for the Gases Investigated With Each Microbubble System

(Values Are Mean ± Standard Error)

Gas	Area Under Time-Intensity Curve					
	Albumin Microspheres (Dog 8103)	Tween-Stabilized (Dog 8090)	Phospholipid-Stabilized (Dog 8088)	Dog 8104		
				Albumin Microspheres	Tween	Phospholipid
Air	2±2	4±2	15±3	NP	NP	NP
SF_6	1±0	30±9	13±3	NP	NP	NP
Perfluoromethane	1±1	58±32	25±3	NP	NP	NP
Perfluoroethane	35±19	126±53	236±142	62±21	247±82	389±96
Perfluoropropane	618±60	2255±186	2806±307	888±279	3750±517	3528±579
Perfluorobutane	1370±305	2009±349	2603±713	NP	NP	NP
Perfluoropentane	10±5	3615±735	2227±512	NP	NP	NP
Hexafluoropropene	NP	NP	NP	NP	9	NP
Octafluoro-2-butene	NP	NP	NP	NP	1	NP
Hexafluoro-1,3-butadiene	NP	NP	NP	NP	24	NP
Octafluorocyclobutane		NP	NP	NP	15	NP

NP = Not performed

The product with the claimed compound perfluoropropane (as well the product with another claimed compound perfluorobutane) show extremely long persistence in all three gas-carrier systems tested (albumin microspheres, Tween® surfactant-stabilized

and phospholipid-stabilized) when compared to the prior art compounds, even the closest one in structure - perfluoroethane. (Products with the third compound - perfluoropentane - showed extremely long persistence in two of the three tests when compared with the prior art). The AUC curves for the claimed compounds showed values of at least between roughly 10-20 times those of the closest structure prior art compound perfluoroethane in all three such systems (with the exception of albumin microspheres containing perfluoropentane).

Figures 5-7 of the Kessler II declaration show plots of the mean AUC values for the analogous fluorocarbon series tested from perfluoromethane to perfluoropentane for three formulations. These figures not only show a statistically significant variation between the prior art gases and those in the claims (again, except for albumin-stabilized microspheres of perfluoropentane), but also show that the values for the prior art gases, perfluoromethane and perfluoroethane, are not statistically different from each other.

The Souza declaration contains an analysis of the differences in the measured AUC areas between the best performing prior art gas (perfluoroethane) and the other gases tested. The prior art gases discussed in this declaration include not only perfluoromethane and perfluoroethane but also air, sulfur hexafluoride and ethane. A summary of those results is expressed below in tabular form

Statistical Significance of the AUC Differences Among Gas-Carrier Systems

Statistically Significant Difference Observed?

Gases Being Compared	Carrier System		
	Surfactant	Phospholipid	Protein Microspheres
A. Prior Art Gases:			
PF-Ethane vs. Air	NO	NO	NO
PF-Ethane vs. SF6	NO	NO	NO
PF-Ethane vs. PF-Methane	NO	NO	NO
B. Claimed Gases:			
PF-Ethane vs. PF-Propane	YES	YES	YES
PF-Ethane vs. PF-Butane	YES	YES	YES
PF-Ethane vs. PF-Pentane	YES	YES	NO

The Souza declaration demonstrates that though there were modest differences between the performances of the prior art gases, these differences were not statistically significant. On the other hand, the differences between the claim designated compounds and the prior art gases were statistically significant, for all three gas-carrier systems of perfluoropropane and perfluorobutane, and for two of the three for perfluoropentane.

The work of Dr. Souza was examined by Dr. Lee, an expert in biostatistics, who states that the Souza analyses are an acceptable basis for determining the statistical significance of the data collected by

Kessler. (Lee declaration, paragraph 4). Dr. Lee further stated (paragraph 5):

> "Under these circumstances, the differences or similarities among gas-carrier systems reported in Mr. Kessler's second declaration provide a proper and reliable (emphasis added) basis for comparing and contrasting such gas-carrier systems."

On the basis of the foregoing, Applicant again 0submits that the combination of references proposed by the Examiner would not have been made by those skilled in the art in 1991, the date when the priority application was filed, and that the claims as currently presented are not prima facie obvious. Applicant further submits that in the event that such claims would be deemed prima facie obvious, the evidence of patentability submitted herewith shows that the current invention is, in fact, not obvious and is patentable.

Double patenting

Claims 30-34 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting over six U.S. patents and six co-pending applications. Applicant is prepared to submit an appropriate terminal disclaimer upon indication of allowable matter. Under the current practice mentioned in the Office Action that statement should be sufficient response to the rejections for the present.

Applicant acknowledges that claims 30 and 31 are provisionally rejected under 35 USC §101 for statutory type double patenting over a co-pending

application, and that such rejection, if still warranted, should be properly withdrawn if it is the only rejection remaining in the application. MPEP §804.

Applicant submits that, on the basis of the arguments presented herein and if necessary, the declarations submitted herewith, the present claims are patentable and requests issuance of a Notice of Allowance.

Respectfully submitted,
LIMBACH & LIMBACH L.L.P.

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Atty Docket No. SNUS-113